Atherosclerosis: role of chemokines and macrophages

Andrew D. Lucas and David R. Greaves

Atherosclerosis is a pathological process that takes place in the major arteries and is the underlying cause of heart attacks, stroke and peripheral artery disease. The earliest detectable lesions, called fatty streaks, contain macrophage foam cells that are derived from recruited monocytes. More-advanced atherosclerotic lesions, called fibro-fatty plaques, are the result of continued monocyte recruitment and smooth muscle cell migration and proliferation. Variable numbers of CD4+ T cells are found in atherosclerotic lesions, and cytokines secreted by T helper 1 (Th1)- or Th2-type cells can have a profound influence on macrophage gene expression within atherosclerotic plaques. This review briefly addresses the key features of macrophage biology and discusses the factors that influence the growth and development of atherosclerotic lesions (atherogenesis). It then considers the potential role of chemokines in mediating monocyte recruitment and macrophage differentiation within atherosclerotic lesions.

Atherosclerosis, a pathological remodelling of the arteries, is a major cause of morbidity and mortality in developed countries and is the underlying basis of myocardial infarction, stroke and peripheral artery disease (Ref. 1). Atherosclerosis can be considered as an unusual form of chronic inflammation occurring within the artery wall (Ref. 2). Fatty streaks, the earliest detectable lesions in atherosclerosis, contain macrophage-derived foam cells that differentiate from recruited blood monocytes. Monocytes are recruited to tissues via constitutive signals and in response to inflammatory mediators. Monocyte recruitment and macrophage differentiation are also a central feature of other important human diseases characterised by chronic inflammation such as rheumatoid arthritis and tuberculosis (Refs 3, 4).

Andrew D. Lucas
Postdoctoral Research Fellow, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK. Tel: +44 (0)1865 285733; Fax: +44 (0)1865 275515; E-mail: andrew.lucas@path.ox.ac.uk

David R. Greaves (corresponding author)
British Heart Foundation Basic Science Lecturer, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK. Tel: +44 (0)1865 285733; Fax: +44 (0)1865 275515; E-mail: david.greaves@path.ox.ac.uk

Laboratory homepage: http://dunn1.path.ox.ac.uk/~greaves/

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More-advanced atherosclerotic lesions, called fibro-fatty plaques, are the result of continued monocyte recruitment, together with smooth muscle cell migration and proliferation (Ref. 5), and can contain CD4+ T cells (Ref. 6). Chemokines or chemoattractant cytokines constitute a family of over 40 different cell-signalling molecules important for constitutive trafficking and recruitment of leukocytes in response to inflammatory mediators (Refs 7, 8, 9, 10). Some chemokines that can act as potent mediators of monocyte migration and macrophage differentiation are expressed in human atherosclerotic lesions (Ref. 11). Indeed, as discussed below, experiments performed with gene-knockout mice lacking macrophage chemoattractant protein 1 (MCP-1) have suggested an important role for the CC chemokine MCP-1 and its specific receptor CCR2 in the initial stages of atherogenesis (Ref. 12).

In recent years, pathologists have advanced the idea of stable and unstable (or vulnerable) atherosclerotic plaques (Ref. 13). Stable plaques are characterised by a thick fibrous cap overlying a plaque that does not contain a cholesterol-rich necrotic core. By contrast, unstable plaques have a thin fibrous cap, contain a higher ratio of macrophages to smooth muscle cells, and have a lipid-filled necrotic core (Ref. 14). Unstable plaques are more likely to rupture, which exposes the thrombogenic core of the lesion to arterial blood. This leads to platelet aggregation and the formation of an arterial thrombus attached to the vessel wall. Thrombus material can break away from the wall and be transported to a distant site (embolism), where it may lead to blockage of smaller arteries. The clinical consequences of arterial thrombosis are heart attacks, strokes and renal disease. Indeed, the majority (~60%) of arterial thrombosis is associated with ruptured atherosclerotic plaques. Another commonly observed feature of atherosclerotic plaques is endothelial cell denudation (plaque erosion) and other changes in the endothelial cells that predispose to arterial thrombosis and its clinical sequelae (Ref. 15).

This article will briefly review some key features of macrophage biology and the factors that influence the growth and development of atherosclerotic lesions (atherogenesis). The potential role of chemokines in mediating monocyte recruitment and macrophage differentiation within atherosclerotic lesions will then considered.

Macrophage biology

Macrophages are bone-marrow-derived phagocytic cells that are important for tissue homeostasis and are found in virtually all tissues of the body. Macrophages also play important roles in the innate and acquired immune responses (Ref. 16). Tissue macrophages are derived from circulating blood monocytes that are recruited to tissues by constitutive or inflammatory signals. Once present in tissues, macrophages can exhibit great variation in morphology and undertake a wide range of physiological functions. For instance, Kupffer cells that line the liver sinusoids are important for the uptake and clearance of modified low-density lipoprotein (LDL) and bacterial endotoxins via macrophage scavenger receptors such as SR-A (Ref. 17). Alveolar macrophages in the lung are important for the clearance of airborne pathogens and excess amounts of surfactant proteins (Ref. 18).

Specific monoclonal antibodies (mAbs) have been developed that allow the detection of both tissue-resident macrophages and macrophages recruited to sites of inflammation and infection. Some of these mAbs (e.g. those against CD68) recognise all macrophages (Ref. 19), while others (e.g. F4/80) recognise only a subpopulation of tissue macrophages (Ref. 20). The heterogeneity in macrophage populations revealed by antibody staining serves to emphasise the diverse physiological roles of macrophages in normal tissues. This diversity is also apparent within macrophage populations present within atherosclerotic lesions. For instance, macrophage-derived foam cells within atherosclerotic lesions express high levels of scavenger receptors, which are important in the pathogenesis of atherosclerosis.

Macrophages are responsible for tissue remodelling during development and wound repair. They secrete many different cytokines, growth factors and proteases that facilitate the remodelling of the extracellular matrix and encourage the recruitment of other cell types such as fibroblasts and smooth muscle cells that are important for wound repair. Key cytokines secreted by macrophages include interleukin 1β (IL-1β), tumour necrosis factor α (TNF-α), IL-10,

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IL-12 and transforming growth factor β (TGF-β). Macrophage-derived growth factors that have significant effects on the pathology of atherosclerosis include fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), which is a potent mediator of smooth muscle cell growth, migration and differentiation, and macrophage colony-stimulating factor (M-CSF), which is an autocrine mediator of macrophage differentiation.

Macrophages can also secrete numerous proteases, protease activators and protease inhibitors in response to physiological signals. Of particular interest in atherosclerosis are members of the matrix metalloproteinase (MMP) family of zinc-containing endopeptidases (Ref. 21). Histochemical and genetic association studies have implicated MMP-1, MMP-2, MMP-3 and MMP-9 as important players in vascular pathology (Refs 22, 23, 24, 25). In addition, activated macrophages secrete tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA); these plasminogen activators activate the serine protease plasmin, which plays a key role in fibrinolysis (the dissolution of blood clots) (Ref. 26).

LDLs and macrophage scavenger receptors

Scavenger receptors are defined by their ability to endocytose modified (e.g. acetylated or oxidised) forms of LDL and were first described by Goldstein and Brown in 1979 (Ref. 27). LDL consists of a cholesterol-rich core packaged by phospholipids and a specific set of apolipoproteins, such as $apoB_{100}$, that target uptake via the LDL receptor (LDLR). LDLs transport cholesterol and triglycerides from the liver to extrahepatic tissues and are taken up and catabolised by specific receptor-mediated endocytosis. Both the lipid and protein moieties of LDL are subject to oxidative damage through the action of free radicals. There is some debate about the precise nature of the chemical modifications to LDL in vivo but it is clear that oxidised LDL is highly atherogenic (Ref. 28). Unlike the classical LDLR, which is downregulated by increasing cellular cholesterol levels, the ability of scavenger receptors to take up modified LDL is not inhibited by increasing cellular cholesterol. This leads to the appearance of macrophage-derived foam cells whose cytoplasm is swollen with lipid droplets. Lipid-laden foam cells are found within the sub-endothelial space of the arteries in fatty streak lesions, which are the first recognisable atherosclerotic lesions, as well as in more-advanced unstable atherosclerotic plaques (Refs 29, 30).

Molecular cloning has identified at least eight different scavenger receptors that can be expressed by macrophages (Refs 29, 30). These receptors have very different structures and can bind and internalise a wide range of polyanionic ligands, including modified forms of LDL (see Table 1). The relative contribution of some scavenger receptors has been assessed in gene-knockout mice and the macrophage scavenger receptors SR-A and CD36 are known to be important in animal models of atherogenesis. Indeed, mice with a disruption in the gene

Table 1. Macrophage scavenger receptors and their ligands[a] (tab001dgw)

Scavenger receptor	Ligands[b]
SR-AI/SR-AII	acLDL, oxLDL, LPS, bacteria
MARCO	Bacteria
CD36	oxLDL
SR-B1	HDL – mediates reverse cholesterol transport
CD68	oxLDL
LOX-1	oxLDL
SR-PSOX	oxLDL
Galectin-3	acLDL, oxLDL, AGE-LDL

[a] Information reviewed in Refs 29 and 30.
[b] Scavenger receptor ligands have been identified by uptake studies in transfected cells and confirmed using blocking antibodies or macrophages of gene-knockout animals.

Abbreviations: acLDL, acetylated LDL; AGE-LDL, advanced glycation endpoint LDL; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LOX-1, lectin-like oxidised lipoprotein receptor 1; LPS, lipopolysaccharide (of Gram-negative bacteria); oxLDL, oxidised LDL; MARCO, macrophage receptor with collagenous structure; SR-PSOX, scavenger receptor that binds phosphatidylserine and oxidised lipoprotein.

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encoding SR-A show a reduced size of atherosclerotic lesions, suggesting a proatherogenic role for SR-A (Ref. 31). Similarly, CD36-knockout mice demonstrate reduced atherosclerotic lesion development when crossed with apolipoprotein E (apoE)-deficient mice and fed a high-fat diet (Ref. 32). Macrophages derived from CD36–apoE double null mice bind and internalise significantly less oxidised LDL than do macrophages from wild-type mice. These results support an important role for CD36 in atherosclerotic lesion development in vivo.

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The development of atherosclerotic lesions in human arteries can be regarded as a modified form of chronic inflammation (Refs 2, 33). The key initial event in this pathology appears to be damage to the endothelial cells of the artery. The exact nature of the endothelial damage is presently unknown but it results in the cell-surface expression by endothelial cells of molecules that mediate leukocyte adhesion, such as intercellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM) (Ref. 34). Following damage to the endothelium, monocytes are recruited into the subendothelial space, where they differentiate into macrophages. These recruited macrophages endocytose modified forms of LDL via scavenger receptors to form foam cells, the hallmark of fatty streak lesions. The lesions develop into fibro-fatty plaques, which contain large numbers of macrophages and some CD4^{+} T cells, and show evidence of smooth muscle migration and proliferation (Fig. 1). In human arteries, these fibro-fatty plaques develop into complex atherosclerotic lesions that are liable to rupture (Refs 35, 36).

Prospective studies have identified several important risk factors for the development of coronary heart disease in human populations. These include smoking, diabetes, hypertension, elevated plasma LDL–cholesterol levels and a family history of heart disease (Ref. 37). In order to define the precise molecular mechanisms by which these environmental and genetic factors influence the process of atherosclerosis, it is essential to study animal models of arterial disease. The most widely used small animal model of atherosclerosis is the apoE-knockout mouse developed in the laboratories of Jan Breslow and Nobuyo Maeda (Refs 38, 39).

Atherosclerosis models

The apolipoprotein apoE is the main protein ligand on the surface of the murine LDL particle, which is recognised by the LDLR. Mice that lack a functional gene encoding apoE have elevated levels of plasma LDL–cholesterol (hypercholesterolaemia). Plasma cholesterol levels can be further elevated by feeding mice a 'western-type diet' containing 21% fat and 0.2% cholesterol. Feeding apoE^{–/–} mice such a high-fat diet results in the development of atherosclerotic lesions in their major arteries, including the aorta and coronary arteries. Another approach to develop a mouse model of atherosclerosis has been to ablate the gene encoding the LDLR, which mimics the situation found in homozygous familial hypercholesterolaemia in humans (Ref. 40). Both apoE^{–/–} and LDLR^{–/–} mice develop atherosclerotic lesions that share many features of early human atherosclerosis and contain large numbers of macrophage-derived foam cells. More-advanced murine lesions also contain CD4^{+} T cells and show evidence of smooth muscle cell migration and proliferation. However, unlike the situation that pertains in human arterial disease, the murine lesions show little or no evidence of atherosclerotic plaque rupture with the resultant thrombo-embolism that characterises human cardiovascular disease. The reason for this lack of progression to more-complex atherosclerotic lesions is not clear.

Although the murine apoE and LDLR gene-knockout models do not recapitulate all the features of human atherosclerosis, they have been widely used to study the effect of specific genes on the earliest stages of atherogenesis. Furthermore, several intervention studies have been performed in apoE^{–/–} and LDLR^{–/–} mice to test the effect of drugs and blocking mAbs on atherosclerotic lesion development. Several studies have revealed an important role for macrophages and macrophage-derived products in atherosclerosis (see Table 2). Ablation of genes involved in various aspects of macrophage differentiation or function result in a decrease in the development of atherosclerotic lesions in these mice, indicating an active role for macrophages in the initiation and growth of atherosclerotic plaques. For an excellent review of genetic modifiers of atherosclerosis in mouse models, see Ref. 41.
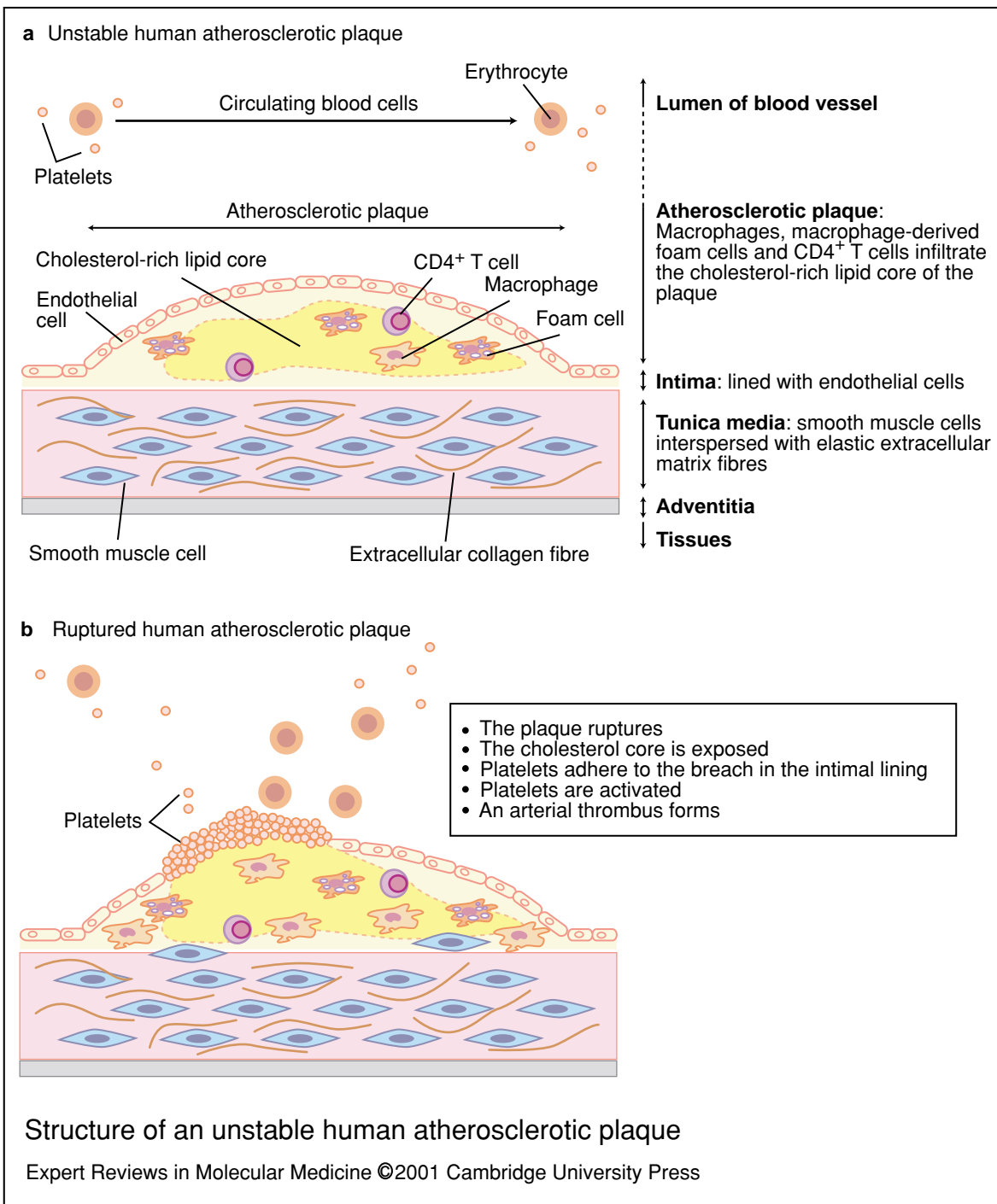
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a Unstable human atherosclerotic plaque

Erythrocyte

Circulating blood cells

Lumen of blood vessel

Platelets

Atherosclerotic plaque

Atherosclerotic plaque: Macrophages, macrophage-derived foam cells and CD4$^+$ T cells infiltrate the cholesterol-rich lipid core of the plaque

Cholesterol-rich lipid core

CD4$^+$ T cell

Macrophage

Endothelial cell

Foam cell

Intima: lined with endothelial cells

Tunica media: smooth muscle cells interspersed with elastic extracellular matrix fibres

Adventitia

Tissues

Smooth muscle cell

Extracellular collagen fibre

b Ruptured human atherosclerotic plaque

Platelets

- The plaque ruptures
- The cholesterol core is exposed
- Platelets adhere to the breach in the intimal lining
- Platelets are activated
- An arterial thrombus forms

Structure of an unstable human atherosclerotic plaque

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Figure 1. Structure of an unstable human atherosclerotic plaque. The normal blood vessel wall comprises an inner layer of endothelial cells (the intima) in contact with the lumen of the vessel, in which the blood circulates, a middle layer of smooth muscle cells and elastic extracellular matrix fibres (the tunica media) and an outer layer of connective tissue (the adventitia) in contact with the tissues. (a) In an atherosclerotic plaque, a cholesterol-rich lipid core forms within the intimal layer and this is infiltrated with cell types such as macrophages, macrophage-derived foam cells (laden with lipid droplets), smooth muscle cells and CD4$^+$ T cells. If the plaque ruptures, as shown in (b), the thrombogenic core of the lesion is exposed to blood in the lumen of the artery. Platelet adhesion and activation initiates the formation of an arterial thrombus **(fig001dgw)**.

Table 2. Genes that modify macrophage biology and atherosclerosis in apoE or LDLR gene-knockout mice[a] (tab002dgw)

Protein encoded by ablated gene	Magnitude of decrease in development of atherosclerotic lesion	Function of deficient protein
M-CSF	5×	Growth factor important for macrophage differentiation
MCP-1	5×	Potent monocyte chemoattractant
CCR2	2–3×	Monocyte/macrophage receptor for MCP-1
CXCR2	~2–3×	Receptor for CXC chemokines
SR-A	5×	Macrophage scavenger receptor for modified LDL
CD36	4×	Scavenger receptor for modified LDL
12/15 LO	2.5×	Generation of inflammatory mediators
CD154	5×	Ligand for the CD40 receptor

[a] For a detailed discussion of the effect of gene ablation on the development of atherosclerotic lesions in apoE$^{-/-}$ and LDLR$^{-/-}$ mice, see Ref. 41.

Abbreviations: 12/15 LO, 12/15 lipoxygenase; CCR2, CC chemokine receptor 2; CXCR2, CXC chemokine receptor 2; LDL, low-density lipoprotein; LDLR, LDL receptor; M-CSF, macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein 1; SR-A, scavenger receptor A.

Chemokines

The chemoattractant cytokines or chemokines are small disulphide-linked polypeptides, typically of 60–70 amino acids in length, and are potent chemoattractants for leukocytes such as T cells, natural killer (NK) cells, monocytes and macrophages (Ref. 42). Although the chemokine supergene family has only been recognised for a decade, it is now known to contain over 40 different members classified into different subfamilies on the basis of conserved structural features.

The CXC (or α-) chemokines have a single amino acid separating the two amino terminal cysteine residues (C1, C2) of the protein, while CC (or β-) chemokines have no amino acid separating the signature C1 and C2 cysteines. Chemokines mediate their effects via interaction with specific chemokine receptors expressed on a wide range of cell types. The CC chemokine receptors are CCR1 to CCR9, and the CXC chemokine receptors are CXCR1 to CXCR6. The fractalkine receptor is CX$_3$CR1 and the lymphotactin receptor is XCR1 (Ref. 43). A list of the currently identified human chemokine receptors and their chemokine ligands is given in Table 3 (Refs 9, 43).

Chemokine receptors are G-protein-coupled receptors (GPCRs) with seven transmembrane (TM7) spanning α-helices. Chemokines bind to their cognate chemokine receptor with high affinity (typically with a dissociation constant of Kd ~1–3 nM). Chemokine receptors are unusual among the many characterised members of the TM7 receptor superfamily in having multiple high-affinity ligands for a single receptor. A good example of this is the CCR4 receptor, which binds the CC chemokines macrophage-derived chemokine (MDC) and thymus- and activation-regulated chemokine (TARC) with high affinity and mediates cellular signalling with nanomolar concentrations of either chemokine ligand (Ref. 44).

Binding of a chemokine to its specific receptor on the cell surface leads to the generation of an intracellular signal via a Gα$_I$-containing G-protein complex and this results in cell chemotaxis towards the source of the chemokine. This process is inhibited by pertussis toxin. In addition to these short-lived signals, some

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Table 3. Human chemokine receptors and their ligands[a] (tab003dgw)

Chemokine receptor	Chemokine ligand[c]	PMN	Mϕ	T cell	Other
CXCR1	IL-8	+	(+)[b]		
CXCR2	IL-8, GRO-α, ENA-78, NAP-2	+	(+)		
CXCR3	IP-10, Mig, I-TAC			+	
CXCR4	SDF-1	+		+	
CXCR5	BCA-1			+	B cells
CXCR6	Bonzo		(+)	+	
CCR1	RANTES, MIP-1α, MIP-1β		+		
CCR2	MCP-1, MCP-2, MCP-3		+		
CCR3	Eotaxin, RANTES, MCP-2			+	Eosinophils
CCR4	MDC, TARC		+	+	
CCR5	RANTES, MIP-1α, MIP-1β		+		
CCR6	MIP-3α				Dendritic cells
CCR7	SLC, MIP-3β				Dendritic cells
CCR8	I-309		+		
CCR9	TECK			+	
CX$_3$CR1	Fractalkine/ CX$_3$CL1		+	+	NK cells
XCR1	Lymphotactin			+	

[a] For further information, see recent reviews Refs 9 and 43.
[b] The plus symbol in parentheses (+) indicates some debate in the literature.
[c] For chemokine nomenclature, see Ref. 9.

Abbreviations: BCA-1, B lymphocyte chemoattractant; ENA-78, epithelial neutrophil-activating peptide 78; GRO-α, growth-related oncogene α; IL-8, interleukin 8; IP-10, interferon-γ-inducible protein 10; I-TAC, interferon-inducible T-cell alpha chemoattractant; Mϕ, macrophage; MCP-1, monocyte chemoattractant protein 1; MDC, macrophage-derived chemokine; Mig, monokine induced by interferon γ; MIP-1, macrophage inflammatory protein 1; NAP-2, neutrophil-activating peptide 2; PMN, polymorphonuclear leukocyte; SDF-1, stromal-cell-derived factor 1; SLC, secondary lymphoid tissue chemokine; TARC, thymus- and activation-regulated chemokine; TECK, thymus-expressed chemokine.

chemokine receptors such as CXCR4 can give rise to prolonged signalling and leukocyte activation via sustained activation of protein kinase B (Ref. 45). Fractalkine/CX$_3$CL1 is a novel chemokine that differs from other chemokines in having three intervening amino acids between the two cysteine residues, resulting in a CX$_3$C motif, and exists as a membrane-bound molecule with the chemokine motif attached to a long mucin stalk (Ref. 46). When cleaved from the cell surface, soluble forms of fractalkine mediate chemotaxis of monocytes, NK cells and T cells (Ref. 46). Immobilised forms of fractalkine have been shown to mediate tight adhesion of cells carrying the CX$_3$CR1 receptor and this adhesion does not require integrins, calcium or an opposing

cell membrane (Ref. 47). Furthermore, fractalkine-dependent firm adhesion of monocytes and T cells can occur under flow conditions (Refs 48, 49).

Many important human diseases, including asthma, arthritis and atherosclerosis are characterised by acute or chronic recruitment of leukocytes from the blood into affected tissues. Chemokines have been implicated as inflammatory mediators in a wide range of pathologies on the basis of studies using clinical material and animal disease models (for reviews, see Refs 50, 51). At first sight, the chemokine network, with its multiple high-affinity ligands and 'promiscuous' receptors, might seem like a cellular signalling system with a great deal of redundancy. However, analysis of chemokine- and chemokine-receptor-knockout mice has revealed a unique, non-redundant role for some chemokines in leukocyte trafficking, inflammation and immunity.

Chemokine- and chemokine-receptor-knockout mice

Initial characterisation of chemokine- and chemokine-receptor-knockout animals has revealed that some ligand–receptor interactions play a key role in the development of the haematopoietic system. One example is the binding of stromal-cell-derived factor (SDF-1) to CXCR4; animals in which genes encoding these proteins are ablated are not viable (Refs 52, 53). Ablation of the murine CXC chemokine receptor CXCR2 causes specific defects in constitutive leukocyte trafficking, with CXCR2 gene-knockout animals exhibiting lymphadenopathy owing to increased numbers of B cells and neutrophils (Ref. 54).

Other chemokine-knockout animals have less obvious phenotypes and exhibit no significant differences in constitutive leukocyte trafficking. Differences in inflammatory cell recruitment are only seen when the animals are immunised, challenged with specific pathogens or subjected to physiological stress. An early example of this was the specific defect in antiviral immunity seen in MIP-1α gene-knockout mice (Ref. 55). Another example, which will be discussed in greater detail in the next section, is deletion of the gene encoding the CC chemokine MCP-1. Mice homozygous for deletion of the gene encoding MCP-1 exhibit no obvious phenotype but, when on a C57/BL6 mouse background and crossed with apoE$^{-/-}$ mice, a significant decrease in the size of atherosclerotic

lesions is observed (Ref. 56). This provides important evidence that MCP-1 plays a non-redundant role in monocyte recruitment and/or macrophage retention in atherosclerotic lesions. A summary of important observations made with chemokine- and chemokine-receptor-knockout animals is provided in Table 4. The list is not exhaustive and new observations will be added as gene-knockout animals are studied more closely in a wider range of disease models.

The role of MCP-1 and CCR2 in atherogenesis

Much of the current interest in the role of chemokines in atherogenesis stems from experiments performed in MCP-1 and CCR2 gene-knockout mice. MCP-1 was among the first of the CC chemokines to be described. MCP-1 (originally called JE) was identified as a PDGF-induced transcript and shown to be a potent chemoattractant for monocytes (Ref. 57). In 1991, MCP-1 expression in human atherosclerotic lesions was clearly demonstrated by in situ hybridisation (Refs 58, 59). Further indirect evidence for MCP-1 being a potential player in atherogenesis came from the demonstration that treatment of human endothelial cells with oxidised LDL induced MCP-1 secretion (Ref. 60). Ablation of the gene encoding MCP-1 in apoE$^{-/-}$ mice results in a marked reduction in the size of atherosclerotic lesions (Ref. 56), strongly suggesting a role for this CC chemokine in atherogenesis.

The TM7 chemokine receptor CCR2, which binds and signals in response to nanomolar concentrations of MCP-1, was cloned in 1994 (Ref. 61). Subsequently, it was shown that cells transfected with CCR2 signal in response to other CC chemokines such as MCP-3 (Ref. 62). In the first paper describing the phenotype of CCR2-knockout mice, it was noted that homozygous CCR2-knockout animals showed reduced clearance of the intracellular bacterial pathogen *Listeria monocytogenes*. Similar to the findings for MCP-1 gene ablation, it was shown that ablation of the CCR2 gene in apoE$^{-/-}$ mice caused a significant reduction in atherosclerosis (Ref. 63). This result has been independently confirmed by other laboratories (Ref. 64) and MCP-1 gene ablation has been shown to reduce the size of atherosclerotic lesions in other transgenic mouse models of atherosclerosis such as mice transgenic for human apoB$_{100}$ (Ref. 65).

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Table 4. Chemokine- and chemokine-receptor-knockout mice (tab004dgw)

Ablated gene	Phenotype	Refs
Chemokine[a]		
MIP-1α	Reduced inflammation following viral infection	55
MCP-1	Reduced atherosclerosis in apoE$^{-/-}$ mice	56, 65
Eotaxin	No effect on eosinophil accumulation in lung inflammation	79
CC chemokine receptors		
CCR1	Reduced NK-cell recruitment	80
CCR2	Reduced atherosclerosis in apoE$^{-/-}$ mice	63, 64
	Increased airway allergic inflammation in response to *Aspergillus*	81
	Reduced airway hypersensitivity following allergen challenge	82
	Increased susceptibility to pulmonary *Cryptococcus* infection	83
	Reduced Langerhans cell migration to draining lymph nodes	84
	Increased severity of experimental glomerulonephritis	85
	Inability to clear *Listeria* infections	86
	Reduced EAE	87
CCR4	Increased resistance to endotoxic shock	88
CCR5	Increased mortality following *Cryptococcus* infection	89
	Reduced clearance of *Listeria* infections	90
CCR6	Reduced humoral immune response to oral antigens	91
CCR7	Altered secondary lymphoid organ structure	92
	Reduced humoral immune responses and contact sensitivity	93
CXC chemokine receptors		
CXCR1	Reduced neutrophil recruitment	50
CXCR2	Lymphadenopathy	54
	Delayed wound healing	94
	Defective acute neutrophil accumulation	95
	Reduce macrophage recruitment in atherosclerosis	72
CXCR4	Non-viable embryos, defects in vasculature	52, 53
CXCR5	Defects in B-cell homing and defective lymph nodes	96

[a] For chemokine nomenclature, see Ref. 9.

Abbreviations: apoE, apolipoprotein E; EAE, experimental autoimmune encephalitis; MCP-1, monocyte chemoattractant protein 1; MIP-1, macrophage inflammatory protein 1, NK cell, natural killer cell.

While it is clear that the MCP-1–CCR2 ligand–receptor combination is playing a role in the initiation of atherosclerosis in mice, it is not clear exactly how MCP-1 expression contributes to atherogenesis. Endothelial cell expression of MCP-1 can be stimulated by treatment with oxidised LDL (Ref. 60), adhesion of activated platelets, homocysteine and the inflammatory cytokine TNF-α. All of these in vitro treatments have physiological relevance for the pathology of atherosclerosis and they appear to act through the transcription factor NF-κB (Ref. 66).

Endothelial cell expression of MCP-1 may be of particular importance in atherogenesis not just

because of the effects of MCP-1 on monocyte chemotaxis but also because of recently described effects of MCP-1 on triggering firm adhesion of monocytes to activated endothelium. Addition of the CC chemokine MCP-1 or the CXC chemokine IL-8 can cause rapid arrest of human monocytes rolling on E-selectin-expressing endothelial cells under flow conditions (Ref. 67). This rapid arrest of rolling monocytes is blocked by antibodies that specifically recognise α and β integrins. These in vitro experiments suggest that MCP-1 could play a doubly important role in monocyte recruitment by mediating both monocyte chemoattraction and firm adhesion of rolling monocytes to activated endothelial cells.

Other biological effects of MCP-1 and CCR2 that might be relevant in the context of atherogenesis are beginning to emerge. One interesting defect in MCP-1-deficient mice is a polarisation of the adaptive immune system towards T helper 1 (Th1)-type immune responses, characterised by the production of interferon γ (IFN-γ) and IL-2, and low levels of expression of the Th2-type cytokines IL-4 and IL-10 (Ref. 68). Thus, high levels of MCP-1 expression within an atherosclerotic plaque containing $CD4^+$ T cells could favour the elaboration of a Th2-type immune response characterised by the expression of Th2 cytokines, which might favour the elaboration of a wound repair response rather than inflammation. It is likely that the multiple effects of MCP-1 on monocyte migration, monocyte adhesion, macrophage differentiation and immune responses account for the growing number of defects observed in experimental disease models in CCR2-knockout mice (see Table 4).

The expression of other CC chemokines in atherosclerosis

Recent gene expression experiments using a DNA microarray of 8600 expressed genes has revealed that the expression of the CC chemokine eotaxin is markedly upregulated in aortic smooth muscle cells treated with TNF-α (Ref. 69). Immunohistochemical analysis of human atherosclerotic lesions showed expression of eotaxin by smooth muscle cells and the presence of $CCR3^+$ cells within macrophage-rich regions of the plaques. Interestingly, the CCR3 chemokine receptor is preferentially expressed on eosinophils, mast cells and Th2-type $CD4^+$ T cells (Ref. 70).

The genes that encode the CC chemokines MDC and TARC and the CX_3C chemokine fractalkine are linked on human chromosome 16q13 (Ref. 71). Expression of MDC and TARC in primary human macrophages is upregulated by the Th2 cytokines IL-4 and IL-13 but not by the Th1 cytokine IFN-γ. Furthermore, analysis of human atherosclerotic lesions by immunohistochemistry has shown that a subset of macrophages within human atherosclerotic plaques express MDC, fractalkine and TARC (Fig. 2 and Ref. 71). It is possible that macrophage expression of these chemokines can be used as a surrogate marker of Th2-type immune responses within human atherosclerotic lesions. However, the mere presence of an inflammatory mediator in a lesion does not mean that it has a significant functional role in the pathogenesis of disease. It will be important to design experiments to critically evaluate the role of CC chemokines other than MCP-1 in atherosclerosis.

The expression of CXC chemokines in atherosclerosis

Early evidence for a potential role for CXC chemokines in atherogenesis came from experiments in which $LDLR^{-/-}$ mice were reconstituted with bone marrow from CXCR2-knockout mice (Ref. 72). The size of atherosclerotic lesions was reduced in mice whose blood contained $CXCR2^{-/-}$ monocytes. The chemokine receptor CXCR2 signals in response to several CXC chemokines, including IL-8 (see Table 3). IL-8 expression in human atherosclerotic plaques has been demonstrated by in situ hybridisation (Ref. 73). Ligands for the peroxisome proliferation associated receptor α (PPARα), including oxidised phospholipids, upregulate endothelial cell expression of IL-8, while ligands for the nuclear receptor PPARγ downregulate IL-8 expression (Ref. 74). Endothelial expression of IL-8 might facilitate firm adhesion of rolling monocytes under flow conditions (Ref. 67) and IL-8 has been shown to decrease macrophage expression of tissue inhibitors of MMPs (Ref. 75). Thus, several lines of evidence suggest that IL-8 expression by cells within the atherosclerotic plaque may be contributing to atherogenesis.

The chemokine receptor CXCR3 has multiple high-affinity CXC chemokine ligands including IFN-γ-inducible protein 10 (IP-10), monokine
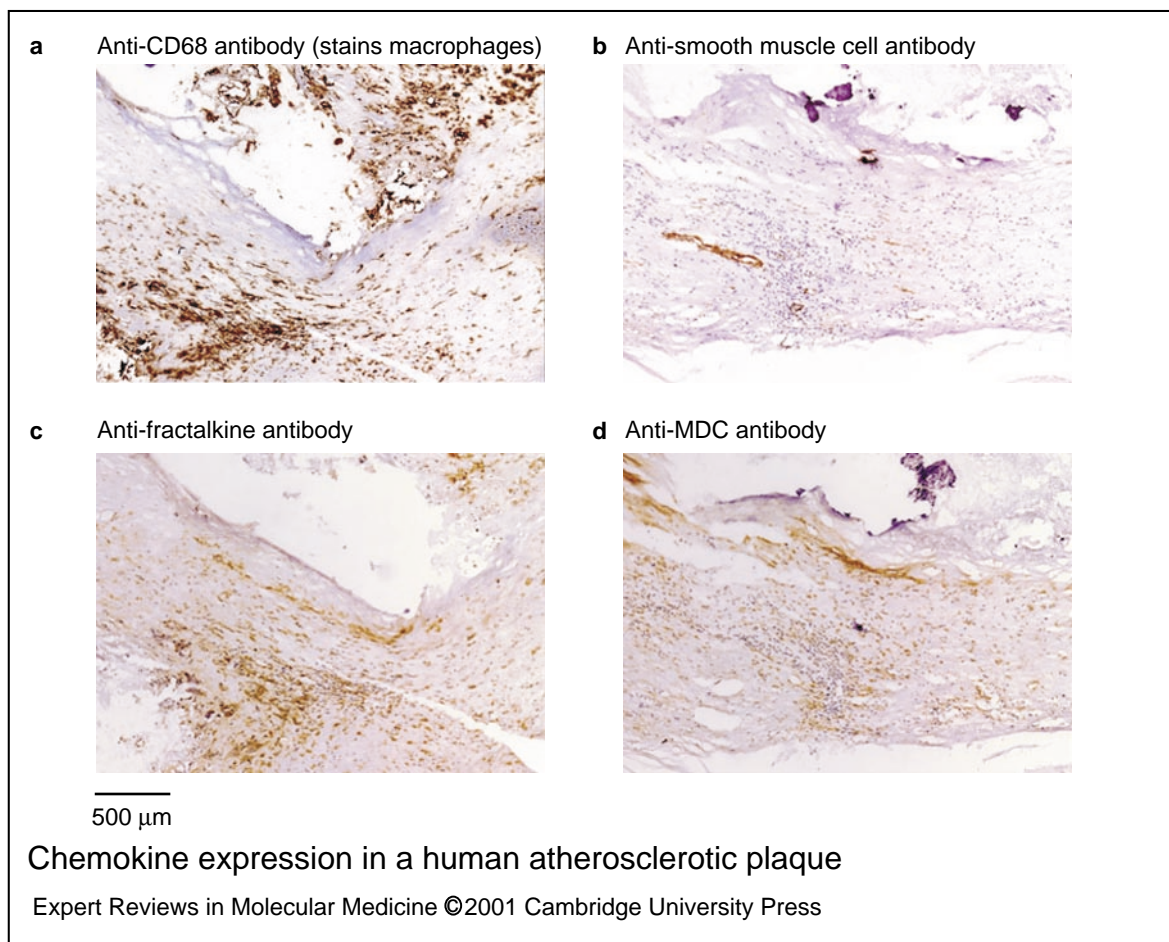
a Anti-CD68 antibody (stains macrophages)

b Anti-smooth muscle cell antibody

c Anti-fractalkine antibody

d Anti-MDC antibody

500 μm

Chemokine expression in a human atherosclerotic plaque

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Figure 2. Chemokine expression in a human atherosclerotic plaque. Immunohistochemistry was performed on serial sections of an atherosclerotic plaque removed from the carotid artery of a 55-year-old man undergoing carotid endarterectomy (surgical excision of the atheromatous tunica intima of an artery). Brown-staining cells are positive for the antigen detected using the indicated primary antibodies. The specificity of staining was confirmed using appropriate control antibodies (not shown). (a) Anti-CD68 antibody stains the many macrophages present in the lesion, whereas (b) the anti-smooth muscle cell antibody reveals that there are very few smooth muscle cells in this atherosclerotic plaque. Staining with specific antibodies that recognise (c) fractalkine and (d) macrophage-derived chemokine (MDC) show that both of these chromosome 16q13-encoded chemokines are being expressed in this lesion. For more details of techniques and controls used in a similar experiment, see Ref. 69 **(fig002dgw)**.

induced by IFN-γ (Mig) and IFN-inducible T-cell alpha-chemoattractant (I-TAC) (see Table 3). All three IFN-γ-inducible CXC chemokines are expressed by endothelial cells and macrophages in human atherosclerotic lesions (Ref. 76). Furthermore, the same atherosclerotic lesions contain T cells expressing the CXCR3 receptor (Ref. 76). Interestingly, the induction of all three CXC chemokines by IFN-γ in endothelial cells can be specifically downregulated by PPARγ ligands (Ref. 77). The role of PPARγ as a potential modulator of inflammation in atherosclerosis is

an interesting area of research in cardiovascular pathology (Ref. 78).

Key questions and future challenges
The aim of this review has been to illustrate the potential importance of chemokines as inflammatory mediators in atherosclerosis. There are clearly many questions concerning chemokines and atherogenesis that remain unanswered.

The dramatic effects of ablating the genes encoding MCP-1 and CCR2 in murine models of

atherosclerosis have shown the importance of this chemokine–receptor combination for monocyte recruitment in the earliest stages of atherogenesis. Small molecule antagonists of CCR2 have been developed and it will be interesting to see if these drugs are effective at reducing atherosclerosis in animal models of cardiovascular disease.

CC chemokines other than MCP-1 are expressed in human atherosclerotic lesions and it will be important to show whether these chemokines play a non-redundant role in atherogenesis. This will require the analysis of appropriate gene-knockout animals or the use of chemokine-binding proteins such as the vCCI protein of vaccinia.

Experimental evidence for the role of CXCR2 in atherogenesis has been presented and immunohistochemistry has shown that several CXC chemokines, as well as the CX_3C chemokine fractalkine, are expressed in human atherosclerotic plaques. Further evidence for the role of these chemokines in human atherogenesis might be provided by analysis of polymorphisms of genes encoding chemokines and chemokine receptors in human populations.

It is important to remember that chemokines are not the only class of inflammatory mediator present in atherosclerotic lesions. However, studying the expression patterns of chemokines in atherogenesis might shed light on important regulatory mechanisms (such as Th1 and Th2 immune responses) that determine the nature of the inflammatory response within the arterial wall.

Perhaps the biggest challenge faced in studying the role of chemokines in atherogenesis is identifying whether chemokine receptors offer new avenues for therapeutic intervention in human cardiovascular disease.

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Further reading, resources and contacts

Reviews

The following three review articles are an excellent starting point for exploring the extensive literature on macrophages, atherosclerosis and chemokines:

Ross, R. (1999) Atherosclerosis – an inflammatory disease. New Engl J Med 340, 115-126, PubMed ID: 99091097

Lusis, A.J. (2000) Atherosclerosis. Nature 407, 233-241, PubMed ID: 20454704

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Glass, C.K. and Witztum, J.L. (2001) Atherosclerosis. The road ahead. Cell 104, 503-516, PubMed ID: 21135273

Textbooks

The following textbooks have good sections on cardiovascular pathology:

Majno G. and Joris, I. (1996) Cells, Tissues and Disease: Principles of General Pathology, Blackwell Science, Cambridge, MA, USA

Woolf N. (2000) Cell, Tissue and Disease, 3rd edn, Harcourt Publishers, Edinburgh, UK

Websites

The Atherosclerosis and Thrombosis Index, a module of the Internet Pathology Laboratory for Medical Education by the University of Utah, provides images of atherosclerotic lesions and has a section devoted to thrombosis and its clinical sequelae, as well as a tutorial on myocardial infarction:

    http://www-medlib.med.utah.edu/WebPath/CVHTML/CVIDX.html

The American Heart Association (AHA) contains many useful sections including the AHA journals and AHA scientific statements. The site has many useful articles for clinicians, researchers and patients:

    http://www.americanheart.org/

The British Heart Foundation (BHF) is the leading UK charity supporting research into all aspects of cardiovascular disease. Their website contains many useful articles for patients and carers:

    http://www.bhf.org.uk/

The Chemokine Website, part of the cytokine family cDNA database, is a useful lexicon of alternative nomenclatures and links to database resources:

    http://cytokine.medic.kumamoto-u.ac.jp/CFC/CK/chemokine.html

The Greaves Lab Website provides an introduction to the research programme of our laboratory in the areas of macrophage gene expression, chemokine biology and atherogenesis:

    http://dunn1.path.ox.ac.uk/~greaves/

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